Remarks

Applicants thank Examiner Nguyen for the helpful telephonic interview held on December 19, 2003. The foregoing amendments address the issues discussed during the interview and are believed to place the claims into condition for immediate allowance or into better condition for consideration on appeal. 37 C.F.R. § 1.116(a). Accordingly, their entry after final rejection is respectfully respected.

Status of Claims

Applicants note that the Amendment, filed October 31, 2003, was not entered. *See*, Advisory Action, page 1.

In the presently proposed Amendment, the status of claims 30, 34, and 36 are identified as "not entered," though their entry is again respectfully requested; claims 33 and 35 are identified as "cancelled"; and claims 14, 28, 29, and 32 are identified as "currently amended" (*i.e.*, they have been amended differently from the [unentered] amendment of October 31, 2003). Support for the amendment to claims 28 and 29 can be found in the present specification at, *e.g.*, page 19, line 22, to page 20, line 1. Support for the amendment to claims 14, 30, and 32 can be found in the present specification at, *e.g.*, page 3, lines 18-21, page 16, lines 26-29, page 27, lines10-20, and page 40, lines 15-27. The amendments to claims 34 and 36 merely adjust claim dependency in view of the cancelled claims. Thus, all of these amendments are believed to introduce no new matter.

Upon entry of the foregoing amendments, claims 14-32, 34, and 36 are pending in the application, with claims 14 and 32 being the independent claims. Based on the above

amendments and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 14-32 remain rejected under 35 U.S.C. § 112, first paragraph, because, according to the Examiner, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. *See,* Paper No. 16, page 3. While the Examiner has indicated subject matter deemed enabled (Paper No. 16, page 2), the Examiner maintains that the specification does not reasonably provide enablement for the claimed method wherein the vector is delivered "by any route of administration, particularly the use of any replication competent viral vectors and/or pathogenic live prokaryotic vectors through a systemic delivery, or the same method using any prokaryotic vector, particularly a prokaryotic expression plasmid vector, any pathogenic, non-attenuated live bacterial vector, or a mammalian artificial chromosome." Paper No. 16, page 3, lines 1-5. Applicants respectfully traverse this rejection.

The point of novelty of the presently claimed invention is that the expression of FPGS in neoplastic cells (at a level higher than the endogenous FPGS level), will enhance the cytotoxic sensitivity of the neoplastic cells to an antifolate drug, such as methotrexate or edatrexate. The particular type of gene delivery system that can deliver the FPGS gene includes viral vectors, non-viral vectors (including cellular vectors), or a hybrid of the two; *see,* specification, pages 15-20, and will, of course vary, based on numerous factors

including, the particular tumor type, the tumor location, the condition of the patient, and the capabilities and/or particular expertise of a given laboratory, to name a few. These factors, as well as others, will be considered by those skilled in the art when determining the best suited gene delivery system.

The above notwithstanding, solely to expedite allowance, and without acquiescing to the propriety of the rejection, claims 14, 28-30, and 32 have been amended herein to accommodate the Examiner's concerns in Paper No. 16, as well as the December 9, 2003 Advisory Action. Accordingly, Applicants believe that this rejection under 35 U.S.C. § 112, first paragraph, has been overcome and should be withdrawn.

Rejections Under 35 U.S.C. § 103

The Examiner maintains the rejection of claims 14-27 and 30-35 under 35 U.S.C. § 103(a), because, according to the Examiner, these claims are unpatentable over Moscow *et al.*, U.S. Patent 5,763,216 ("Moscow"), in view of Roy *et al.*, *J. Biol. Chem. 272*: 6903-6908 (1997) ("Roy"), Kim *et al.*, *J. Biol. Chem. 268*:21680-21685 (1993) ("Kim"), and Garrow *et al.*, *Proc. Nat'l. Acad. Sci. 89*:9151-9155 (1992) ("Garrow") for the reasons set forth previously in Paper No. 13. *See*, Paper No. 16, page 9, first paragraph.

In Paper No. 13, the Examiner stated:

> It would have been obvious and within the scope of skills for an ordinary skilled artisan to modify the method of Moscow *et al.* by direct delivery of a non-viral (plasmid) or viral vector comprising a DNA sequence encoding human FPGS into neoplastic cells *in vivo* that have acquired resistance to methotrexate and other classical folate analogues in order to reverse the resistance of MTX or other antifolate drugs in these neoplastic cells, so that to enhance the efficacy of

> conventional anti-folate drug therapy in light of the teachings of Roy *et al.*, Kim *et al.*, and Garrow *et al.* It is noted that as defined by the present application, a neoplastic cell is a cell whose normal growth control mechanism is disrupted thereby providing the potential for uncontrolled proliferation (citations omitted). As such, tumor cells resistant to MTX or other antifolate drugs would be encompassed within the scope of neoplastic cells of the instant invention. Furthermore, by reversing the resistance to MTX and other antifolate drugs in the tumor cells, the cytotoxic sensitivity of the tumor cells to an antifolate drug is in effects [sic] enhanced.

Paper No. 13, pages 23-24.

The Examiner also stated:

> One of ordinary [skill] in the art would have been motivated to carry out the above modification because Moscow *et al.*, Roy *et al.* and Kim *et al.* recognize that decreased folylpolyglutamate synthetase is a factor contributing to the resistance of tumor cells to methotrexate or other antifolate drug treatment, and by increasing the exogenous expression of FPGS in MTX or other antifolate resistant tumor cells, the sensitivity to antifolate drugs of the treated tumor cells would be enhanced and thereby enhancing the efficacy of traditional antifolate chemotherapy. (Emphasis in original).

Paper No. 13, page 24, lines 7-14.

Finally, the Examiner stated:

> One of an ordinary skilled artisan would have a reasonable expectation of success because Kim *et al.* clearly teach that lowered FPGS activity and decreased polyglutamylation of antifolates are thought to be general mechanisms by which cancer cells become resistant to a wide range of antifolates, and that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing high levels of human ... FPGS are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). Furthermore, Roy *et al.*clearly show that L1210 tumor cells resistant to methotrexate or edatrexate have lowered FPGS activity. Therefore, the

> claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary. (emphasis in original).

Paper No. 13, pages 24-25. Applicants respectfully traverse the rejection.

Applicants will now discuss the teachings of each reference individually, followed by a discussion concerning why a legally sufficient *prima facie* case of obviousness has not been established.

The primary reference, the Moscow patent, relates solely to the human reduced folate carrier (RFC) gene, expression vectors comprising the RFC gene, as well as the use of such vectors to restore methotrexate (MTX) sensitivity to MTX-resistant, transport deficient, cancer cells. *See*, Moscow patent, Abstract. The MTX-resistant cancer cells studied in the Moscow patent contained markedly decreased expression of the RFC gene compared to the parental cell line. *Id.*, at sentence bridging cols. 15 and 16. According to the "Summary of the Invention" section of the Moscow patent, "The purpose of this [RFC] gene therapy is to restore RFC activity, and thereby re-establish the sensitivity of these cancer cells to MTX drug treatment." *Id.*, at col. 2, lines 6-12.

As discussed in the "Background of the Invention" section of the Moscow patent, RFC is one of two mechanisms by which animal cells uptake folate from their environment. Accordingly, the RFC mechanism is involved in facilitating MTX (folate antagonist) uptake, and although other genes besides RFC may be involved in the development of MTX-resistance (including overexpression of dihydrofolate reductase, alteration of dihydrofolate reluctance affinity for MTX, decreased FPGS, and decreased thymidylate synthase levels), according to the Moscow patent, "decreased MTX uptake is the principal characteristic in many MTX-resistant cell-lines." Moscow Patent, col. 1, 2nd paragraph. Thus, a fair reading of the Moscow patent, as a whole, relates to: (1) the RFC gene (and not the FPGS gene), as

the RFC gene is capable of facilitating MTX uptake; and (2) the restoration of RFC activity as a means to re-establish MTX sensitivity to MTX-resistant, transport deficient cancer cells.

Significantly, the Moscow patent, as a whole, teaches RFC gene transfer at the exclusion of all other known genes involved in folate metabolism (see list in prior paragraph), thereby "teaching away" from using other genes (such as FPGS) that may be involved in the acquisition of MTX resistance. As correctly acknowledged by the Examiner, the Moscow patent does *not* specifically teach the use of a vector comprising FPGS nor the enhancement of cytotoxic sensitivity to an anti-folate drug, such as MTX, when FPGS is expressed in neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells. In fact, as stated above, in the Moscow patent, RFC activity is being restored in order to re-establish MTX sensitivity. This is in contrast to the present case, wherein a totally different gene, FPGS (that is expressed at an endogenous level by the neoplastic cell) is being expressed at a level higher than the endogenous level in order to enhance MTX activity (rather than restore it).

As discussed below, Roy, Kim, and Garrow do not remedy these fundamental deficiencies in the Moscow patent.

Roy teaches that L1210 cell variants which express either decreased or increased levels of FPGS (compared with parental lines) display corresponding differences in resistance to folate analogues. According to the Examiner, Roy teaches that, *in vitro*, L1210 tumor cells resistant to MTX have a decrease in the rate of FPGS mRNA transcript formation, resulting in lower FPGS activity (page 6907, col.2, first full paragraph).

Kim teaches that mutant Chinese hamster ovary (CHO) cells, lacking FPGS activity, exhibit increased sensitivity to pulses of MTX in cell culture after being transfected with an

FPGS expression cassette. Thus, Kim teaches that FPGS cDNA transfection restored cytotoxic sensitivity of FPGS-deficient CHO cells to methotrexate. Kim does not teach the transformation or transfection of neoplastic cells. In addition, Kim does not teach the transformation or transfection of cells which express an endogenous level of FPGS activity (like neoplastic cells), nor show that such transformation or transfection with an FPGS gene can enhance the neoplastic cell's cytotoxic sensitivity to an anti-folate drug.

Garrow teaches the cloning of a human FPGS. Garrow teaches that transfecting the cloned FPGS into mutant CHO cells lacking FPGS activity restored the ability of the transfected mutant cells to grow in culture in the absence of purines and thymidine. Garrow does not teach the delivery of a vector comprising a nucleotide molecule that encodes an FPGS into *neoplastic cells*, nor does Garrow teach the treatment of cells expressing FPGS with an antifolate drug. Further, Garrow doesn't teach or suggest that transformation or transfection of neoplastic cells with an FPGS gene can enhance the neoplastic cell's cytotoxic sensitivity to an anti-folate drug.

Rejection of claimed subject matter as obvious under 35 U.S.C. § 103 in view of a combination of references requires (1) consideration of whether prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or carry out the claimed process, and (2) whether the prior art would also have revealed that such a person would have reasonable expectation of success; both suggestion and reasonable expectation of success must be found in the prior art, not in Applicant's disclosure. *See, In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Further, all claim limitations must be taught or suggested by the prior art. *In re Royka*, 180 U.S.P.Q. 580 (CCPA 1974).

Applicants maintain that the Examiner has not established a *prima facie* case of obviousness because he has not pointed to anything, in the cited references or in the body of knowledge generally possessed by those skilled in the art, that would suggest the modification or combination of the references necessary to arrive at Applicants' claimed invention. Moreover, there is not a reasonable expectation of success simply because the Moscow patent utilized a different gene in a gene transfer context. Although it might have been obvious for the skilled artisan to try and see if cytotoxic sensitivity of neoplastic cells to anti-folate drugs could be enhanced by introducing an FPGS gene, such does not give rise to a *prima facie* case of obviousness. *See, In re Geiger*, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987) and *In re Fine*, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

Applicants presently pending claims are not directed to the restoration of cytotoxic sensitivity to neoplastic cells that are resistant to methotrexate or other folate analogues. Rather, the presently amended claims recite: "A method of enhancing the cytotoxic sensitivity of neoplastic cells to an antifolate drug, said neoplastic cells expressing an endogenous level of folylpolyglutamyl synthetase (FPGS), said method comprising:

(a) delivering directly to said neoplastic cells a vector, said vector comprising a DNA sequence encoding folylpolyglutamyl synthetase (FPGS), operably linked to a promoter, *wherein said FPGS is expressed in said neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells;*

(b) treating the neoplastic cells in step (a) with an antifolate drug that is polyglutamated by said FPGS; and

(c) *enhancing the cytotoxic sensitivity of said neoplastic cell to said antifolate drug."* (Emphasis added).

In rationalizing the rejection, the Examiner has relied on the theory in Kim that "[l]owered FPGS activity may be a general mechanism by which cells can become resistant to a wide range of antifolates." This theory, however, does not teach or suggest that in the context of vector-mediated gene therapy, the elevation of FPGS activity beyond the endogenous level characteristic of a particular tumor cell, will enhance their cytotoxic sensitivity. Also, this theory does not provide the requisite motivation to modify or combine the cited references in order to arrive at the presently claimed invention, especially when the Moscow patent "teaches away" from using genes other than RFC. That is, the Moscow patent acknowledges that several different genes may be involved in the development of MTX-resistance (including decreased RFC, overexpression of dihydrofolate reductase, alteration of dihydrofolate reluctance affinity for MTX, decreased FPGS, and decreased thymidylate synthase levels), but that "decreased MTX uptake is the principal characteristic in many MTX-resistant cell-lines." Moscow Patent, col. 1, 2nd paragraph. Thus, the Moscow patent teaches how to improve the ability to transport MTX into cells (*i.e.*, uptake) by increasing the expression of RFC, rather than exploiting any other gene involved in folate metabolism. Clearly, this can be viewed as "teaching away" from using any other gene (besides RFC) since the inventors of the Moscow patent are teaching how to increase MTX uptake (by increasing RFC expression) in order to improve MTX resistance. The Moscow patent does not teach or suggest up- or down- regulating any other gene involved in folate metabolism, though they do acknowledge them.

Applicants note that the claims are not dependent on any particular mechanism of action, nor do they necessarily require that the neoplastic cells be MTX resistant. The point of novelty of the currently claimed invention is the teaching and demonstration that, in the

context of vector-mediated gene therapy, the elevation of FPGS activity beyond the endogenous level characteristic of a particular tumor cell, will augment their cytotoxic sensitivity.

As discussed above, Kim shows that vector-mediated transfection of FPGS cDNA can restore FPGS activity and reintroduce cytotoxic sensitivity into variant CHO cells that express *no endogenous* FPGS activity. In contrast, the currently claimed invention recites that the FPGS is transferred to neoplastic cells which have endogenous FPGS activity. Applicants have shown that elevation of FPGS activity via vector-mediated gene therapy, *beyond the endogenous level characteristic of most tumor cells*, will augment their cytotoxic sensitivity. That is, the issue of whether tumor cells, already expressing FPGS, can be imbued with enhanced antifolate sensitivity after FPGS gene delivery has not been previously addressed by any of the cited art, taken alone or in combination. The limitations of the claimed method have not been met.

Applicants contend that the Examiner has not provided a sufficient explanation as to why a person skilled in the art would have been motivated to modify the teachings of Kim or Garrow such that the cloned FPGS gene is delivered, not to a mutant Chinese hamster ovary cell, but to a *neoplastic cell*.

In each cited reference, the gene being transfected is not present in the cell being studied. The cells are either FPGS deficient or RFC deficient. None of the cited references teach or suggest that FPGS can successfully be transferred to neoplastic cells which display *some* endogenous level of FPGS activity. Applicants have shown that elevation of FPGS activity via vector-mediated gene therapy, *beyond the endogenous level characteristic of most tumor cells*, will augment their cytotoxic sensitivity. That is, the issue of whether tumor

cells, already expressing FPGS, can be imbued with enhanced antifolate sensitivity after FPGS gene delivery has not been previously addressed by any of the cited art, taken alone or in combination. The limitations of the claimed method have not been met.

Applicants direct the Examiner's attention to page 40, lines 16-27, of the specification, where the teachings of Roy and Kim were discussed and distinguished from the present invention. The Applicants stated:

> It has been shown that downregulation of a tumor's FPGS activity via mutation leads to antifolate resistance (Pizzorno, G., *et al., Cancer Research 48*:2149 (1988); Roy, K., *et al., Journal of Biological Chemistry 270*:26918-26922 (1995); Roy, K., *et al., Journal of Biological Chemistry 272*:6903-6908 (1997); Takemura, Y., *et al., British Journal of Cancer 75 (suppl. 1)*:31 (1997)), and that transfection of mutant CHO cells lacking FPGS activity with a plasmid bearing the FPGS cDNA enhances their susceptibility to MTX pulses (Kim, J.S., *et al., Journal of Biological Chemistry 268*:21680-21685 (1993)). It was unclear, however, whether increasing the FPGS expression of a tumor cell line **already displaying intermediate FPGS enzyme activity** would enhance the cell line's MTX susceptibility. Emphasis added.

In the Advisory Action, at page 2, the Examiner stated that "none of the pending claims recited that a tumor cell displays intermediate FPGS enzyme activity." In response, Applicants respectfully submit that although the pending claims do not *verbatim* recite that the tumor cells have "intermediate FPGS enzyme activity," the proposed claims recite that the neoplastic cells "express an endogenous level of folylpolyglutamyl synthetase (FPGS)," and that the "FPGS is expressed in said neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells." Clearly, one skilled in the art would appreciate the correspondence between the specification and the claims.

In summary, Applicants submit that there is no motivation to modify or combine the cited references in order to arrive at the claimed invention, especially in view of the Moscow

patent "teaching away" from using other known genes involved in folate metabolism. Further, there is no expectation of success of achieving Applicants' invention simply because gene transfer was successful with a totally different gene (*i.e.*, RFC). The combination of references provides nothing more than an "invitation to try" the combination proposed by the Examiner. Thus, at best, the Examiner is using an inappropriate "obvious to try" standard. *See, In re Fine*, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Accordingly, a *prima facie* case of obviousness has not been established. Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

Next, the Examiner rejects claims 14 and 28-29 under 35 U.S.C. § 103(a), because, according to the Examiner, these claims are unpatentable over the Moscow patent, in view of Roy, Kim, and Garrow, as applied to claims 14-19, 22, 25-28, 31 and 32 above, and further in view of Pawelek *et al.*, *Cancer Research 57*:4537-4544 (1997)("Pawelek"). *See,* Paper No. 13, pages 25-26. Applicants respectfully traverse the rejection.

The teachings of all references, except Pawelek, are discussed and distinguished above. Pawelek teaches the use of an attenuated *Salmonella* as an anticancer vector for gene delivery into tumor cells. The Examiner contends that it would have been obvious for a skilled artisan to modify the combined teachings of Moscow, Roy, Kim, and Garrow for delivering a DNA sequence encoding human FPGS into tumor cells resistant to methotrexate and other folate analogues by using an attenuated *Salmonella* as an anticancer gene delivery vector, as taught by Pawelek.

Applicants respectfully submit that Pawelek does not remedy any of the fundamental defects of the prior rejection, see *supra*. Accordingly, this rejection is improper and should be withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite allowance of this application, the Examiner is invited to telephone the undersigned directly at (202) 772-8637. Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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